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RESEARCH ARTICLE

Evaluation of Chicken Oil as a Dietary Energy Source in Caged Layers and its Impact on Egg Production, Egg Quality and Intestinal Morphology

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ABSTRACT

The purpose of this experiment was to investigate how chicken oil affects production performance, egg quality and gut morphology of layers. A total of 120 Lohmann Single Comb White Leghorn commercial layers (25-week-old) were randomly assigned to four treatments having three replicates each (10 layers per replicate) under a completely randomized design. Chicken oil was used in feed @ 0.0, 1.5, 3.0 and 4.5% in various treatment groups (20 weeks duration). Chicken fat obtained from broiler skin is a very good economical source of energy in the animal feed industry. Results of the present study indicated that feed intake significantly increased up to level of 3% chicken oil whereas 4.5% chicken oil group had higher body weight, egg weight and egg mass. Weekly egg production and feed conversion ratio/dozens of eggs were significantly increased in 1.5% chicken oil treatment group. Laying hens fed 1.5% chicken oil diet had better egg specific gravity and eggshell thickness. Eggshell weight, Haugh unit score, yolk weight, albumin weight and yolk color improved by addition of 4.5% chicken oil in layer diet. Adding 3% chicken oil improved albumin height, yolk height and yolk diameter. Hens raised on 4.5% chicken oil showed increased villus height in ileum while duodenum and jejunum showed lower villus height. However, 3% chicken oil increased crypt depth in duodenum and ileum. Villus height to crypt depth ratio was decreased in all parts of intestine. It was concluded that addition of chicken oil in layer diet @ 1.5% improved hen production performance, egg quality without negative effect on gut morphology.

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INTRODUCTION

Fats and oils are frequently used in poultry diets to meet chickens high energy needs (Gao *et al.*, 2022). Addition of lipids to diet has many benefits, including increased feed palatability, better dust management in sheds, feed mills and improved absorption of fat-soluble vitamins (Saleh *et al.*, 2021). Additionally, fat increases chicken feed effectiveness and slows feed's passage through chickens' digestive system, giving nutrients more time to be absorbed (Chwen *et al.*, 2013). According to National Research Council (NRC), specific level of fat in layer feed should be between 1.5% and 4.5%.

Most popular energy sources in laying hen diets are oils and fats, which have several advantages, such as boosting animal immunity, improving feed intake and reducing morbidity (Gopi, 2013). Adding oils to the diet of laying hens boosts egg production, feed efficiency and digestibility of other dietary components (Pérez-Bonilla *et al.*, 2011; Khatun *et al.*, 2018). Generally, less oil is added to laying hens' feed because of their distinct physiological makeup from broilers' and because they are more susceptible to disorders of lipid metabolism. Right amount and kind of oil addition is crucial for laying hens' production efficiency, lipid metabolism and egg quality.

Cereal grains and lipid sources both are used in poultry feed industry to fulfill energy requirements of birds. However, grain prices increased tremendously as they are an integral part of human diet. There is need of alternate inexpensive feed materials which can reduce overall feed cost. Nowadays nutritionists are more focused to search cheaper sources of energy (Ravindran *et al.*, 2016; Attia *et al.*, 2020). Therefore, to fulfill energy requirements layers feed includes byproducts of animal origin such as lard, tallow and chicken oil because vegetable oils and grains are expensive sources of energy (Kim *et al.*, 2019).

Chicken oil (CHO) may be used by chicken species at a faster rate as compared to tallow and lard oil (Zhang *et al*., 2011; Saleh *et al*., 2021). CHO is produced by the extraction of chicken fat through rendering. It is reported that broiler skin adipose tissues contain about 30% oil (Lin and Tan, 2017). It is one of the most unsaturated animal fat because it has the greatest amount of oleic acid (49%) and least linoleic acid contents (22%) (Suresh *et al*., 2019). Although CHO is frequently utilized in feed mills due to their high nutritional value. Chicken oil can be used safely when processed at high temperature (Saleh *et al*., 2021). However, limited information is available regarding use of different levels of CHO and its effect on layer production and gut morphology. Therefore, current study was conducted to investigate effect of various dietary CHO levels in laying hens on egg production, egg quality traits and gut morphology (26 to 45 weeks of age).

MATERIALS AND METHODS

Experimental animals, design and treatments: A total of 120 Lohmann Single Comb White Leghorn commercial layers (25-week-old) were kept in cages and randomly assigned to four treatments having three replicates each (10 layers/replicate). Chicken oil used in this experiment was extracted from chicken fat by rendering process and was commercially purchased. Chicken oil was used @ 0.0, 1.5, 3.0 and 4.5% in treatment groups. Hens were fed various diets for 20 weeks. Birds received 16 hours of light daily. Proximate analysis of diets was performed prior to start of experiment by method outlined by AOAC (2010).

Data collection, sampling and analysis

Laying performance: Birds were weighed separately on arriving at the farm. A weighed amount of feed was given daily to layers. Weekly feed intake was calculated from quantity of feed offered and any leftover feed at the end of respective week. Hen day egg production (HDEP) and hen housed egg production (HHEP) were calculated by following formula as described by Mangnale *et al.* (2019).

Hen Day Egg Production = (Number of eggs produced/ week) / (Number of live hens) x 100

Hen House Egg Production = (Number of eggs produced/ week) / (Number of housed hens) x 100

Additionally, feed conversion ratio (FCR) / per dozen eggs was computed.

Egg quality: Three eggs per replicate were taken weekly to test egg weight (EW), specific gravity (SG), eggshell weight (ESW), eggshell thickness (EST), yolk color (YC), yolk height (YH), albumin height (AH), Haugh unit score (HU), yolk diameter (YD), yolk weight (YW), albumin weight (AW) and yolk index (YI). Digital electronic balance was used to determine egg weight, albumin weight and yolk weight (Model, JJ3000B). Yolk color fan was used to note YC intensity. EST was measured by eggshell thickness meter (P-1 Model, Meg Co Ltd., Ozaki, Japan). Egg SG was determined by the method described by Crosara *et al.* (2019). A digital vernier caliper (0-150 mm) was used to measure YD. YD (mm) was measured when jaws touched yolk's margins. Formula below was used to compute YI:

$$\text{Yolk index} = \frac{\text{Yolk height (mm)}}{\text{Yolk diameter (mm)}}$$

AH and YH were measured using an egg meter (OSK 13471 Model, Ogawa Seiki, Co Ltd, Japan). Egg meter nob was placed in center of albumin and yolk to determine their height. Nob of meter was lowered until it contacted albumin and/or yolk surface (Dilawar *et al*., 2021). HU score was calculated by using following formula:

$HU = 100 \log H + 7.37 - 1.71W^{0.37}$

Where

HU = Haugh Unit

W = Egg weight (g)

H = Height of albumin (mm)

Gut morphology: Three hens per replicate were slaughtered at the end of experiment to determine gut morphology. Tissue samples from the intestine were preserved in a 10% neutral buffered formalin solution. Paraffin sectioning method was used to extract tissue from duodenum (10 cm distal to the duodeno-gizzard junction), jejunum (5 cm proximal to Meckel's diverticulum) and ileum (5 cm prior to the ileo-cecal junction). Sections were created using a semi-automated rotary microtome (AMOS Scientific AEM-450, Austria) and stained using Hematoxylin and Eosin staining technique. Independent images were collected for gut morphological investigations, villus height (µm) and crypt depth (µm) were measured. Villus height: crypt depth ratio was also calculated by the method described by Kiczorowska *et al*. (2016). The images were taken with Labomed LX400 (Labo America Inc. USA) and were processed with Image software (National Institutes of Health, USA).

Statistical design: One-way ANOVA under CRD with GLM procedure of SAS (SAS Institute Inc., Cary, NC) was applied to analyze data collected and means were separated through Duncan's Multiple Range test at probability level of 5%, considering each pen as an experimental unit.

RESULTS

Laying performance: Laying hen performance was significantly improved (P<0.05) by inclusion of CHO in layer diet (Table 2). Highest BW was recorded in 4.5% CHO treatment group while highest FI was recorded in 3% CHO treatment group. Average egg weight was higher in 4.5 % CHO group. However, CHO 1.5% have significantly higher (P<0.05) egg mass (EM), HDEP and HHEP. FCR per dozen eggs was higher in the control group.

Egg quality: Egg quality traits were significantly affected (P<0.05) by inclusion of CHO in layer diet (Table 3). Addition of CHO to layer diet had a significantly (P<0.05) higher EW, ESW, AW and YW. Higher level of dietary inclusion of CHO (4.5%) had larger eggs and increased YC, HU, AW, YW and YI. Addition of 3% CHO in layer diet significantly (P<0.05) increased SG, EST, YD, AH and YH.

Gut morphology: Effect of CHO on different segments of small intestine (duodenum, jejunum and ileum) was seen by changes in their morphometry by measurement of villus height (VH), crypt depth (CD) and villus height to crypt depth (VH:CD) ratio. In this study, CHO addition to layer

Table 1: Ingredient composition of experimental diets.

	Treatments			
Ingredients (%)	T_1	T_2	T_3	T_4
Maize	52.2	60.7	54.3	45.5
Soybean meal 44%	23.2	19.4	18.5	15.7
Limestone	8.55	8.40	8.56	8.75
Rice Polishing	0.00	0.12	4.00	10.0
Rice tips	10.4	0.00	0.00	0.00
Canola meal 36 %	0.00	4.47	6.00	10.0
Guar meal	3.00	3.00	3.00	3.00
Dicalcium Phosphate	1.54	1.52	1.62	1.43
Chicken oil	0.00	1.50	3.00	4.50
Sodium bicarbonate	0.33	0.47	0.47	0.47
NaCl	0.27	0.30	0.24	0.24
Premix *	0.22	0.22	0.22	0.22
DL-Methionine	0.74	0.15	0.15	0.15
Lysine sulphate 55%	0.05	0.06	0.06	0.06
Phytase 10000 C	0.005	0.005	0.005	0.005

Table 2: Nutrient composition of experimental diets.

	Treatments			
Nutrients (%)	T_1	T_2	T_3	T_4
Metabolizable energy (kcal/kg)	2750	2750	2750	2750
Crude Protein	16.0	16.0	16.0	16.0
Calcium	3.75	3.75	3.75	3.75
Avail. Phosphorus	0.39	0.39	0.39	0.39
Lysine	0.82	0.82	0.82	0.82
Methionine	0.40	0.40	0.40	0.40
Meth + Cyst	0.68	0.68	0.68	0.68
Arginine	1.08	1.08	1.08	1.08
Tryptophan	0.18	0.18	0.18	0.18
Threonine	0.60	0.60	0.60	0.60

T_1: diet containing 0%, chicken oil, T_2: diet containing 1.5% chicken oil, T_3: diet containing 3% chicken oil, T_4: diet containing 4.5% chicken oil.

Table 2: Effect of chicken oil on production performance of caged layers.

	Treatments[3]					
Parameters	T_1	T_2	T3	T4	SEM	P-value
FI (g)	825.6^{ab}	825.2^{ab}	826.6^{a}	825.1^{c}	0.892	0.041
BW (g)	1500.00^{b}	1493.33^{c}	1462.22^{d}	1584.44^{a}	0.779	0.030
WEP (%)	05.68^{c}	06.28^{a}	05.87^{b}	05.87^{b}	0.893	0.024
EW (g)	57.02^{b}	56.77^{c}	57.39^{b}	58.19^{a}	1.149	0.040
AEM (g)	45.19^{d}	50.90 ^{a}	49.54^{c}	50.04^{ab}	0.682	0.036
FCR	01.80^{a}	01.60^{c}	01.70^{b}	01.71^{b}	0.672	0.032
HDEP (%)	81.53^{d}	88.83^{a}	84.04^{b}	83.83^{c}	2.669	0.027
HHEP (%)	81.17^{a}	88.83^{c}	83.83^{b}	83.83^{b}	2.25	0.022

Values within the same row which have different superscripts letter are significantly different (P<0.05): FI: feed intake, BW: final body weight, WEP: weekly egg production, AEW: average egg weight, EM: egg mass, FCR: feed conversion ratio, HDEP: hen day egg production, HHEP: hen house egg production.

Table 3: Effect of chicken oil on egg quality characteristics of caged layers

	Treatments[3]					
Parameters[2]	T_1	T_2	T3	T4	SEM	P-value
SG	1.09^{b}	1.10^{a}	1.09^{b}	1.10^{a}	1.829	0.043
EST (mm)	0.35^{c}	0.39^{a}	0.37^{ab}	0.36^{c}	1.459	0.031
ESW (g)	8.34^{cd}	8.41^{b}	8.30^{c}	8.48^{a}	2.069	0.021
YH (mm)	17.93^{c}	18.63^{b}	19.93^{a}	18.00^{b}	1.259	0.029
AH (mm)	7.77^{c}	8.58^{ab}	8.62^{a}	7.69^{c}	2.359	0.012
HU	88.15^{b}	86.27^{c}	86.88^{c}	89.34^{a}	1.509	0.012
YD (mm)	39.73^{c}	39.78^{c}	40.20^{a}	40.05^{b}	0.839	0.03
YW (g)	16.14^{b}	16.38^{b}	16.52^{a}	16.52^{a}	2.249	0.046
AW (g)	32.54^{ab}	31.99^{c}	32.57^{ab}	33.19^{a}	1.549	0.026
YC	9.73^{c}	9.90^{ab}	9.80^{c}	9.98^{a}	1.839	0.107
YI	0.45^{a}	0.44^{b}	0.45^{a}	0.45^{a}	1.649	0.027

Values within the same row which have different superscripts letter are significantly different (P<0.05): EW: egg weight, SG: specific gravity, EST: eggshell thickness, ESW: eggshell weight, YH: yolk height, AH: albumin height, HU: Haugh unit, YD: yolk diameter, YW: yolk weight, AW: albumin weight, YC: yolk color, YI: yolk index.

diet had a significant (P<0.05) effect on VH, CD and VH: CD ratio in different parts of small intestine as shown in Table 4. VH in duodenum and jejunum portions of small intestine in CHO supplemental groups considerably decreased when compared to control group. CD significantly (P<0.05) increased in small intestine's duodenum and ileum part but decreased in jejunum. It was discovered that the addition of 3% CHO in layer diet had the highest CD in duodenum and ileum. VH:CD ratios in all areas of small intestine were reduced by CHO addition to layer diet. Layers fed 4.5% CHO had lowest VH:CD ratio in duodenum and jejunum.

Table 4: Effect of chicken oil on gut morphology (um) in caged layers.

Variables	Treatments					
	T1	T2	T3	T4	SEM	P-value
VH (um)						
Duodenum	1087.27^{a}	872.89^{d}	1037.72^{ab}	888.25^{c}	0.779	0.03
Jejunum	1181.01^{a}	399.50^{c}	453.14^{b}	243.95^{d}	1.269	0.035
Ileum	1151.08^{c}	1077.51^{d}	1169.32^{b}	1209.25^{a}	0.699	0.032
CD (um)						
Duodenum	67.36^{d}	112.63^{c}	141.37^{a}	125.41^{b}	1.649	0.014
Jejunum	171.78^{a}	86.57^{b}	80.23^{c}	83.70^{b}	2.019	0.035
Ileum	154.17^{c}	156.28^{c}	291.41^{a}	241.57^{b}	2.999	0.015
VH: CD						
Duodenum	16.13	7.75	7.34	7.08	1.783	0.012
Jejunum	6.88	3.91	5.65	2.91	0.832	0.023
Ileum	7.47	6.89	4.01	5.01	0.966	0.014

Values within the same row which have different superscripts letter are significantly different (P<0.05): VH: Villus height, CD: crypt depth, VH: CD: villus height to crypt depth.

DISCUSSION

Production performance: Layers receiving 4.5% CHO showed higher BW. Higher fat content of feed might be the cause of increased BW in layers and lower egg production while 1.5% CHO level resulted in increased egg production. Similar findings were reported by Yarmohammadi *et al*. (2020) and Poorghasemi *et al*. (2013) who found that increased body weight in broilers by oil addition in feed. While 1.5% CHO level resulted in increased egg production. These findings are consistent with those of Zhouyang *et al*. (2021) who observed that adding fat at a lower level 1.5% to layer diets increased egg production. Oils in feed improved nutrient absorption in diets and may have generated some favorable conditions for an improvement in egg production (Ravindran *et al*., 2016).

EW and EM were higher by the addition of CHO in layer diet. Addition of oils greatly enhanced EW on its own without influencing amount of dietary metabolizable energy (Zhouyang *et al*., 2021). This outcome is consistent with the findings of Zaazaa *et al*. (2022) who claimed that addition of various types of oils in layer feed had increased EW. In addition, birds receiving 1.5% CHO showed higher FI and laid more eggs than in control group. As a result, higher EM of layers receiving treated ration may possibly be caused by higher FI as well as more effective feed utilization by these birds, as narrated by Dai and Bessei (2009). Similar findings have been observed by Kim *et al*. (2019) who studied the addition of varied quantities of dietary lipids on increased EM, production performance and egg quality in laying hens. FCR per dozens of eggs was significantly decreased by addition of CHO in cage layer diet. Similar results regarding FCR have also been reported by Gao *et al*. (2020) due to inclusion of palm oil in layers significant effect on FCR. Better nutrient digestion and absorption, which ultimately may have led to improved egg production.

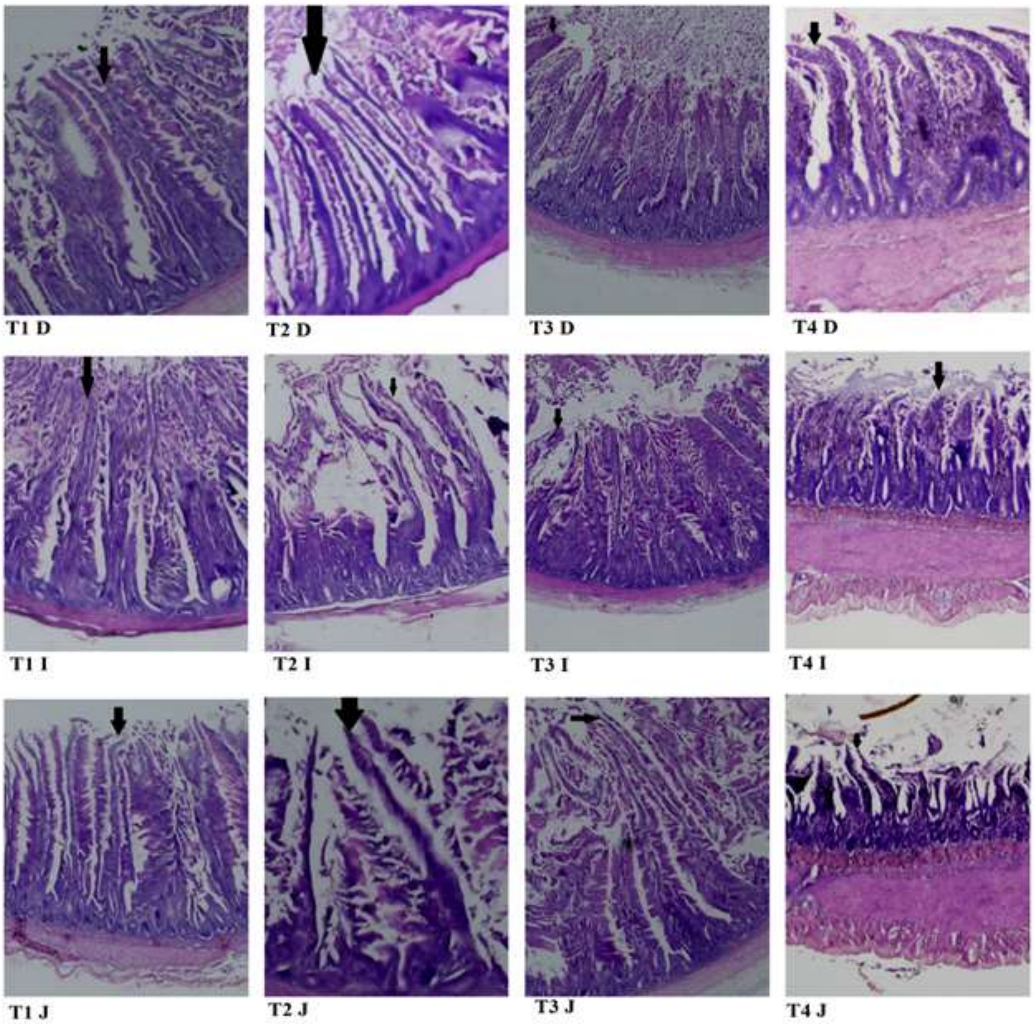

Fig. 1: Gut morphology of small intestine: T1 D (control dedendum villus height) T2 D (treatment 2 duodenum villus height)) T3 D (treatment 3 duodenum villus height) T4 D (treatment 4 duodenum villus height): T1 I (control ileum villus height) T2 I (treatment 2 ileum villus height) T3 I (treatment 3 ileum villus height) T4 I (treatment 4 ileum villus height): T1 J (control Jejunum villus height) T2 J (treatment 2 Jejunum villus height) T3 J (treatment 3 Jejunum villus height) T4 J (treatment 4 Jejunum villus height).

Egg quality: Egg SG, ESW and EST were improved significantly in this study. However, by increasing CHO amount in layer diet and producing bigger eggs ultimately increased ESW. Present study results are in line with the findings of Gao *et al*. (2022), Kim *et al*. (2019) and Zaazaa *et al*. (2022) who reported that various oils and fat sources in layer diets have an effect on ESW. However, higher quantity (4.5%) of CHO in layer diet resulted in decreased EST. Decreased EST might be due to poor calcium availability to layers because in lipid digestion, calcium serves a crucial dual role by increasing the removal of long-chain fatty acids from the oil-water interface and reducing their bio accessibility (Zhang *et al*., 2008). Similar findings showed that increasing the level of canola oil in layer diets decreased EST (Zhang *et al*., 2011; Gul *et al*., 2012). The relationship between SG and EST is strong. Because of this, measurements of SG were used to assess eggshell quality. Results of this study are consistent with those reported by Güçlü *et al*. (2008) who investigated the impact of various dietary fat levels on SG of quail eggs and discovered that adding oils to the diet had a substantial impact on SG of quail's eggs. HU score, AL, YH, AW, YW, YC and YI were significantly affected by CHO addition in cage layer diet. HU score, AH, and YH are indicators of egg freshness. Albumen and yolk quality deteriorates as there is increase in egg storage time and temperature. Fresh eggs were used in current study. However, results of this study showed that adding CHO @ of 3.0% to diet increased AH and YH. Similarly, YW, AW and HU increased by increasing the level of CHO (4.5%) in layer diet. CHO contains 37% linoleic acid (Ravindran *et al*., 2016), an increased HU score of treated groups may be caused by higher linoleic acid content in feed.

YD significantly increased by the addition of 3% CHO in layers diet. Addition of oil to layer feed might have enhanced diameter of egg yolks because unsaturated fatty acids are readily absorbed by hen bodies entering blood vessels and subsequently directly deposited on egg yolk (Senkoylu *et al*., 2004). Similarly, there was a significant variation in YC and YI of eggs produced by addition of CHO. These results are consistent with what Kim *et al.* (2019) who added animal fat in layer feed and observed that addition of oil in layer diet improve the YC.

Gut morphology: Animal health is largely determined by their digestive systems and avian condition may be inferred from anatomy of their guts. In digestion and absorption of feed nutrients, the small intestine, particularly duodenum and jejunum play a crucial role. Growth rate is directly correlated to the bird intestinal health, which results in better absorption and utilization of the feed ingredients (Jazi *et al*., 2018). According to Zeitz *et al*. (2015) longer villus and lower crypt depth resulted in a higher mucosal surface area and increased digestive efficiency. In this study villus height increased by the addition of 4.5% CHO in ileum part of the small intestine. These results are in line with Perveen *et al*. (2020) who observed that addition of oil in broiler diet significantly increased villus height in duodenum part of small intestine. CD was also increased up to level of 3% CHO in duodenum part of intestine in layers. These findings consistent with Stamilla *et al*. (2020) observed that villus height and crypt depth increased by the addition of essential oil in broiler diet. CD is responsible for the renewal of villi (Rebolé *et al*., 2010). The addition of CHO in layer feed reduced VH in the duodenum and jejunum and CD in jejunum and ileum part of small intestine. This may be because CHO caused damage of intestinal villi, which reduces surface area for absorption of nutrients.

Conclusions: It was found that inclusion of chicken oil improved laying hen performance only up to 1.5% level, but egg quality traits may be improved by adding higher level (4.5%) of chicken oil in layer feed. However, it was found that intestinal health is affected as the chicken oil level was increased in layer feed. So, it was concluded that chicken oil can be added into the layer diet @ 1.5% to enhance egg production, egg quality without negative effect on gut health.

Authors contribution: MY designed project and finalized manuscript. SL conducted a research trial and analyzed data. FA applied statistics and interpreted results. MKS does gut morphometry and review manuscript.

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